METHOD OF DIAGNOSIS

The present invention relates to a method for the diagnosis or detection of a predisposition to developing cardiac hypertrophy, for monitoring the development of cardiac hypertrophy and for monitoring the efficiency of therapy for cardiac hypertrophy and a kit for the same.

Cardiac hypertrophy is an important adaptive response of the heart to injury or to an increased demand for cardiac output or when there is increased resistance to blood flow. Following long-standing hypertension, the heart adapts through the activation of a hypertrophic response that is characterised by the reactivation of genes normally expressed during foetal heart development, an enlargement of myocardial cells and an accumulation of sarcomeric proteins in the absence of cell division.

High blood pressure, or systemic hypertension, has long been recognised as a major cause of morbidity and mortality in animals and man. In man prolonged systemic arterial hypertension has been shown to be associated in a wide range of non-fatal and fatal events such as myocardial infraction (heart attack), cerebrovascular accident (stroke), heart failure and renal failure (kidney damage) (Perea, G, 1955, J. Chronic Dis. 1: 33-42). For many years untreated hypertension has been known to increase cardiovascular and cerebrovascular mortality (MacMahon, S. *et al.*, 1990, Lancet 335: 765-74). Over the last twenty years studies with a variety of classes of drug which reduce blood pressure have been shown to reduce the morbidity and the mortality due to stroke and heart disease.

Some epidemiological investigations such as the Framingham Study (Levy, D. et al., 1990, N. Eng. J. Medicine 322: 1561-6 and Kannel, W.B. 1991, J. Hypertension 9(2): 53-9) show that the presence of cardiac hypertrophy of heart muscle induced by sustained hypertension was associated with a poor prognosis, The risk associated with left ventricular hypertrophy has been emphasised by more recent work. Table 1

below summarises a number of known factors and the risks attributable to each, of subsequently having a myocardial infarction.

Table 1

Risk Ratios of Cardiovascular Disease

AGE ADJUSTED RISK RATIO	MEN	WOMEN
Raised cholesterol	1.7	1.4
Hypertension	2.2	2.5
Diabetes	2.2	3.7
Left Ventricular Hypertrophy	4.7	7.4
Cigarette Smoking	1.7	1.2

Kannel, W.B. 1991 *supra.*

Not every person with hypertension will develop cerebro- or cardio- vascular problems. The therapeutic benefit and the cost benefit ratio of treating subjects with mild to moderate hypertension without any additional risk factors has been under question. Indeed it is anticipated that British treatment guidelines will be revised to so that only those with mild to moderate hypertension with an additional risk factor or evidence of declared target organ damage will receive treatment.

It is now appreciated that the presence of one or more additional risk factors such as left ventricular hypertrophy identifies those hypertensive patients who should be treated. Ultimately, the object of therapy is to reduce morbidity and morality associated with hypertension and this may come through an approach which reduces in a targeted manner a number of risk factors. Therefore, the importance of stopping smoking gained importance as part of the intervention regimen and is justified by the fact that it is the factor associated with the second highest risk (hazard ratio). Furthermore the last decade has seen the development of highly effective and well tolerated agents to reduce blood cholesterol and studies on primary and secondary

prevention of myocardial infarction have shown that a reduction of serum cholesterol has major therapeutic benefit. This is in addition to the management of raised blood pressure with anti-hypertensive drugs.

As the presence of Left Ventricular Hypertrophy (LVH) is the highest risk factor according to Kannel, W.B. 1991 *supra.* it is desirable for all mild hypertensives to be assessed at regular intervals so as to predict and assess the development of LVH and thus the need for therapy to prevent the onset of cerebro- or cardio- vascular problems.

Other diseases associated with cardiac hypertrophy include a range of diseases of the heart and lungs which include pulmonary hypertension, cystic fibrosis and chronic obstructive airways disease, that result in hypertrophy of the right side of the heart. Once hypertrophy of the right ventricle has occurred, most of these diseases are associated with a considerable increase in morbidity and mortality. It would therefore be desirable to monitor the development of hypertrophy of the right ventricle and to assess the adequacy of management of a predisposing condition.

Current methods available for detecting cardiac hypertrophy involve the use of electorcardiography and imaging techniques such as echocardiography and magnetic resonance scanning. There are disadvantages associated with these techniques in that electrocardiography only detects severe forms of cardiac hypertrophy and echocardiography and magnetic resonance imaging are expensive techniques not immediately available to all physicians dealing with hypertension.

Studies have shown (JAMA 1979, 242: 2562-71) that the electrocardiogram only identifies some 3-8 % of patients at the time of diagnosis of hypertension as having cardiac hypertrophy and thus the technique does not provide for effective diagnosis. Echocardiography and magnetic resonance scanning have shown that the incidence of left ventricular hypertrophy at diagnosis of hypertension is of the order of 20-60 % in unselected hypertensive patients (Savage, D.D. et al., 1979, Circulation 59: 623-32).

However these imaging techniques are expensive and require that the patient attend a hospital, for the most part, whereas the majority of hypertension is treated in the community. Another drawback of imaging techniques is that they are able to identify only established hypertrophy and are unable to identify patients in whom the changes that will result in hypertrophy have been initiated.

It is an object of the present invention to provide a method for identifying those patients with cardiac hypertrophy and those who are likely to develop the condition, allowing early therapy to be commenced.

According to the present invention in a first aspect there is provided a method for diagnosing or detecting a predisposition to cardiac hypertrophy comprising assaying a sample of human bodily fluid *in vitro* for the level of cardiotrophin-1 contained therein.

The invention is based on the inventor's finding that cardiotrophin-1 levels are elevated in blood samples of patients who have been shown by classical methods such as electrocardiography and echocardiography to have cardiac hypertrophy as compared to persons not having cardiac hypertrophy

Cardiotrophin-1 (CT-1) is a cytokine of the family that includes interleukin-6 (IL-6), leukaemia inhibitory factor (LIF), ciliary neurotropic factor (CNTF), oncostatin-M (OSM) and interleukin-11 (IL-11). CT-1 is widely but not universally expressed in adult mouse tissues including heart, kidney and liver. *In-vitro* studies have demonstrated that levels of CT-1 in tissue samples from mice rise as a result of a hypertrophic response in cardiomyocytes and suggest that CT-1 activates myocardial cell hypertrophy (Wollert, K.C. *et al.*; J. Biol. Chem. Vol. 271, No. 16, pp9535-45, 1996). A more recent study (Ishikawa, M. *et al.*, J. Hypertension 1999, 17: 807-16) concluded that expression of CT-1 mRNA is increased in the early stage of ventricular hypertrophy (in rats) and remains elevated after the hypertrophy is established. A

further conclusion of this study was that it is unlikely that CT-1 plays a mechanistic role in the development and maintenance of LVH in rats.

For a gene or gene product to be suitable as a marker for a disease or predisposition for a disease it is important that the gene product is produced at the onset of, and during the complete cycle of disease progression and that the level of production does not fluctuate greatly. It is known that both α-adrenergic transmitters and endothelin are expressed in cardiac hypertrophy. However as plasma levels of these vary greatly in response to a whole range of factors over short periods of time, the use of these factors as indicators of cardiac hypertrophy is not viable. It is also important that the marker is elevated or reduced only in response to the onset or development of the disease under study and not due to other conditions. For example one suggested marker for cardiac hypertrophy is atrial natiuretic factor (ANF). The problem with ANF as a marker of cardiac hypertrophy is that ANF is raised in a number of other conditions, such as heart failure. In consequence, its use as a reliable marker of cardiac hypertrophy is flawed.

The prior art contains little evidence that CT-1 plays a role in cardiac hypertrophy and no evidence that it is involved in cardiac hypertrophy in man and no suggestion that this cytokine would be released from human tissues if present and provide a suitable marker of cardiac hypertrophy in bodily fluid samples.

Furthermore, in man, ventricular hypertrophy is related to the pressure independent trophic effects of a number of humoral factors including hormones such as adrenaline and angiotensin II. These hormones in man influence, not only the histological pattern observed in hypertensive hypertrophy but also the degree of hypertrophy. This is in contrast to the changes seen in animal models of hypertension such as the SHRsp (Pennica, et al., 1995, PNAS 92: 1142-6) and would suggest that different mechanisms are involved in the onset and development of ventricular hypertrophy between humans and other animal models, such as the rats and mice of the prior art.

It is a preferred embodiment of the invention that the diagnosis or detection of a predisposition to cardiac hypertrophy is determined by comparison of basal CT-1 levels in a human bodily fluid sample from a subject unaffected by cardiac hypertrophy and the level of CT-1 in a bodily fluid sample of a subject under test.

CT-1 is present at a basal level in human bodily fluid samples of subjects unaffected by cardiac hypertrophy. This serves as background. It is this basal level which serves as the comparison with the level of CT-1 in the bodily fluid of a subject under test. Elevated CT-1 levels as compared to the basal level are indicative of a predisposition to cardiac hypertrophy or onset and development of cardiac hypertrophy. The determination of actual hypertrophy may then be confirmed by classical techniques such as electrocardiography and imaging techniques.

The method according to the present invention has several advantages over the prior art:

The method may be used to diagnose or detect a predisposition to cardiac hypertrophy of both the right ventricle (right venticular hypertrophy – RVH) or the left ventricle (left ventricular hypertophy – LVH).

The cause of ventricular hypertrophy may be evident before detection of the hypertrophy or ventricular hypertrophy will be detectable at a time when the patient presents with an underlying problem.

LVH is a high risk factor for subjects with hypertension to develop cardiac problems. Diagnosis or detection of a predisposition to LVH in hypertensive persons allows such persons to be treated for hypertension to prevent the onset or development of LVH and thus a high risk of developing cardiac problems.

RVH occurs in a range of disease of the heart and lungs which include pulmonary hypertension, cystic fibrosis and chronic obstructive airways disease. Diagnosis of RVH therefore provides an indicator of severity of such a condition.

As CT-1 expression is elevated at the initiation of biochemical changes which result in cardiac hypertrophy, the method may be used to detect patients at risk of developing cardiac hypertrophy before any irreversible damage has occurred, i.e. before the onset of cardiac hypertrophy.

The assay may be easily carried out on samples of bodily fluid and there is therefore not the need for a person to visit a hospital having a certain imaging machine to carry out a test for cardiac hypertrophy. The assay may be carried out in a doctor's surgery, by a healthcare worker, and it is even envisaged that the assay may be developed so that it can be produced as a test kit which is sufficiently simple to be used in the home by the subject at risk of cardiac hypertrophy. The method allows a relatively inexpensive and quick test for cardiac hypertrophy as compared to the classical techniques presently in use.

The main advantage of the method of the present invention is its accuracy. The electrocardiogram has been shown to correctly identify only 3-8 % of patients at the time of diagnosis of hypertension as having cardiac hypertrophy and thus the technique does not provide for effective diagnosis. Echocardiography and magnetic resonance scanning have shown that the incidence of left ventricular hypertrophy at diagnosis of hypertension is of the order of 20-60 %. It is thought that the method of the present invention may prove to diagnose even more subjects with cardiac hypertrophy and also before the condition develops.

The method of the present invention may be used to monitor the onset and progression of cardiac hypertrophy. Elevated CT-1 levels as compared to normal are indicative of the initiation or onset of cardiac hypertrophy.

The method of the invention may be used to monitor the progression of cardiac hypertrophy by comparing the CT-1 level in a test sample with that of a previous sample from the same subject. Elevated levels in the subsequent sample are indicative of developing cardiac hypertrophy. Reduced levels in the subsequent sample are indicative of receding cardiac hypertrophy or optimisation of therapy.

The method according to the present invention may be used to monitor the efficacy of therapy for cardiac hypertrophy. By carrying out repeated test over time the trend in CT-1 levels may be monitored. A reduction in CT-1 levels over time is indicative of an effective therapy.

It is preferred that the method is performed yearly, more preferably, every six months, even more preferably quarterly and most preferably every six weeks. The number of assays taken per year would vary depending on the degree of risk associated with the subject and the results of any previous assay.

The method according to the present invention may be carried out on whole blood, plasma, serum, urine, tears, sputum, saliva or synovial fluid samples.

According to a preferred embodiment of the invention the method comprises an *in vitro* assay arranged to detect CT-1 protein or specific fragments thereof. Preferably this *in vitro* assay comprises radio immuno assay or enzyme-linked immunosorbant assay (ELISA). Preferably a specific binding partner for CT-1 or fragments thereof can be used quantitatively to show levels of CT-1 in a sample. The specific binding partner is preferably functional, producing a label, or has a label attached thereto to show the presence of levels of CT-1. Examples of specific binding partners for CT-1 include the CT-1 receptor and anti-CT-1 antibodies, although other suitable specific binding partners will be apparent to those skilled in the art.

Preferably antibodies raised against the whole CT-1 protein or specific fragments thereof are used in immuno assays as specific binding partners for CT-1. A suitable antibody for use in the assay is rabbit IgG to full length human CT-1.

Other ways of determining levels of CT-1 protein in a sample are by molecular weight or charge. Chromatography on a porous carrier or SDS PAGE may be used to show levels of CT-1 in a sample due to the distance travelled along the carrier. Isoelectric focussing may be used to identify CT-1 due to its charge.

According to an alternative embodiment of the invention the method comprises an *in vitro* assay arranged to detect CT-1 nucleic acid or fragments thereof. Preferably this *in vitro* assay comprises hybridisation, sequencing or amplification techniques such as PCR.

As when assaying for both the CT-1 protein and nucleic acid it is important to show the level of CT-1 it is preferable that the *in vitro* assay is quantitative. The reagents and vessels necessary for assaying CT-1 levels may be provided in a kit.

It is known that CT-1 expression is not localised to the heart uniquely in mice but is expressed in other mouse tissues including the kidney and liver. To increase the accuracy of the method according to the first aspect of the invention a further *in vitro* assay for an additional marker is carried out.

Suitable markers of cardiac function include ANF, oncostatin M, ciliary neurotrophic factor and leukaemia inhibiting factor.

The main importance of diagnosing cardiac hypertropy is due to the implication that LVH in addition to hypertension can lead to cardio- and cerebro- vascular problems.

Accordingly the second aspect of the invention provides the use of the method of the first aspect of the invention to determine subjects who should be treated for hypertension.

LVH is a high risk factor for heart problems in combination with hypertension and therefore if elevated levels of CT-1 are found in a sample of bodily fluid that person should be treated for hypertension to prevent the development of heart problems.

According to the third aspect of the invention, the efficacy of treatment for hypertension may be monitored by comparing the CT-1 level in a test sample with that of a previous sample from the same subject. A reduction in CT-1 levels over time is indicative of an effective therapy.

The present invention will now be further described with reference to the following non-limiting example.

EXAMPLE

A protocol for performing the method according to the first aspect of the invention involves competitive ELISA.

Briefly the competitive ELISA involves binding by passive adsorption , the antigen (purified human CT-1) overnight. Plasma samples may be added with the competeing antibody which is rabbit IgG to human CT-1 or the plasma sample could be added for a period of incubation before the competing antibody. An enzyme-labelled antibody is then added for colour development.

The assay may be further improved by the used of monoclonal antibodies for increased sensitivity and a reduction in the number of steps.

Summary of steps:

(i) Passive adsorption of antigen	-add purified CT-1 to plate overnight
(ii) Wash	
(iii) Add samples/standards	-addition of plasma
(iv) Addition of competing antibody	- add rabbit IgG to human CT-1
(v) Wash	
(vi) Add enzyme labelled antibody	- add anti-rabbit conjugated IgG
(vii) Wash	
(viii) Add substrate	- addition of colour development system
(ix) Distinguish colour change	- read absorbance

A further assay which has been shown to determine the presence of CT-1 levels in human plasma is described in Talwar, S. et al., Biochem. & Biophys. Res. Comms. 261 567-571 (1999), which was published after the priority date of this application. Talwar et al. describes a non-radioactive immunoluminometric sensitive and specific assay for CT-1 based on a competitive ligand binding principle.

The chemiluminescent label 4-(2-succinimidyl-oxycarbonylethyl)phenyl-10-methyl-acridinium-9-carboxylate fluorosulfonate was used to label a peptide representing a domain in the middle section of CT-1. Assay of this domain of CT-1 (amino acids 105-120) in patients with heart failure revealed elevated CT-1 values compared to normal controls.

The assay for CT-1 according to Talwar et al. is assumed to have utility according to a preferred embodiment of the present invention.